Anomeric Nature of the D-Mannose Residues in the *Salmonella typhi* and *S. strasbourg* Lipopolysaccharides

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Lipopolysaccharides (LPS) from *Salmonella* bacteria groups E_1, E_2, and E_4, contain β-D-mannopyranose residues, as demonstrated by fragmentation analysis,[1-3] and these are believed to be associated with the presence of the common O-factor 3.[4] *S. strasbourg* (9,46), group D_2, cross-reacts with the anti O 3 factor system,[5] thus indicating that the D-mannopyranose residues in the corresponding LPS are β-linked in disagreement with a previous suggestion.[6]

In our studies on the LPS from *S. typhi* (group D_1) and *S. strasbourg* (group D_2), the anomeric configurations of the D-mannopyranose residues were not determined. The results indicated, however, that they should be α-linked in the *S. typhi* LPS in disagreement with previous results,[9] and thus have the same configuration as the corresponding residues in the groups A [10] and B [11] LPS. In order to settle these questions, the anomeric configuration of the sugar residues in the *S. typhi* I. S. 59 and *S. strasbourg* I. S. 627 LPS have now been determined.

A new technique [12] has been used, which is based upon the observation by Angyal and James [13] that acetylated β-glycosides (equatorially oriented aglycone in the most stable chair form) are readily oxidized by chromic acid in acetic acid, but the corresponding α-glycosides are fairly stable.

The LPS were dissolved in 0.2 % aqueous acetic acid and kept at 100° for 1 h. The recovered LPS, with a reduced lipid content, were acetylated with acetic anhydride-pyridine in formamide. The acetylated polysaccharides were recovered by gelfiltration on a (lipophilic) Sephadex LH 20 column, and then treated with chromic acid in acetic acid at 50°. Samples were withdrawn after 1 and 2 h, and the sugar composition of their hydrolysate was determined (Table 1).

On oxidative treatment of the D_2 polysaccharide, the D-mannopyranose residues are rapidly oxidized, indicating that they have the β-configuration.

Both polysaccharides contain α-tyvelopyranose residues (3,6-dideoxy-α-D-*arabino*-hexopyranose), linked to D-mannose at C-3. Part of these (about 50 %) disappeared during the reaction, but as the D-mannopyranose residues were unaffected, on oxidation of the D_1 LPS, this must be due to oxidation, presumably to 5-keto-hexonic acid esters, and not to acid hydrolysis.

Similar results were obtained when the oxidation of abequopyranosides (3,6-dideoxy-D-*xylo*-hexopyranoside) was investigated.[12]

From these results, combined with those from previous studies,[7,8] it is possible to propose complete structures for the oligosaccharide repeating units in the O-specific side chains of the two LPS and at the same time justify the presence of O-factor 3 in *Salmonella* group D_2.

Experimental. The polysaccharides (10 mg) were dissolved in 0.5 ml $HCONH_2$. Acetic anhydride and pyridine (1 ml of each) were added and the solution kept at room temperature overnight. After evaporation, the remaining solution was added to a column (27 × 3 cm) of Sephadex LH 20 (lipophilic), which was then irrigated with a mixture of chloroform:acetone (2:1).

The fully acetylated polysaccharides (checked with IR) were dissolved in 0.3 ml of acetic acid, 15 mg of CrO_3 was added and the mixture was treated in an ultrasonic bath at 50°.

Samples of 0.15 ml were removed and partitioned between water and chloroform. The chloroform phases were evaporated and the recovered materials were subjected to sugar analysis as described earlier.[14]

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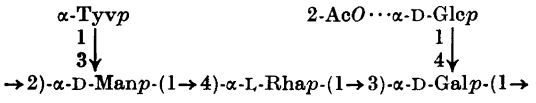
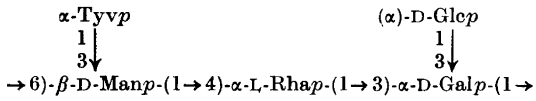


Group D_1



Group D_2

Table 1. Sugar composition of original and oxidized polysaccharides.

Strain	Oxidation time, h	Relative proportions			
		D-Rha	D-Man	D-Gal	D-Glc
S. typhi	0	20	21	23	17
S. typhi	1	19	21	20	16
S. typhi	2	16	20	19	16
S. strasbourg	0	18	19	27	9
S. strasbourg	1	18	3	18	3
S. strasbourg	2	17	1	18	3

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